V. RANGASWAMY* & T. PULLAIAH*: **Studies in the embryology of *Gnaphalium polycaulon* Pers. (Compositae)**

V. ランガスワミ*・T. プライア*: *Gnaphalium polycaulon* (キク科) の花粉, 胚嚢, 胚形成

Gnaphalium, a large genus of about 200 species belonging to the tribe Inuleae of the family Compositae, is cosmopolitan in distribution (Willis 1973). A perusal of the available literature on the embryology in the family Compositae reveals that the genus *Gnaphalium* is embryologically unknown (Pullaiah 1984). Hence the present investigation has been undertaken. *Gnaphalium polycaulon* Pers. is a slender, spreading, soft white woolly annual herb.

Material and methods The material has been collected by one of us (T. P.) in Bukkapatnam RF. Heads of different stages of development have been fixed in formalin-acetic-alcohol (F. A. A.). Customary methods of dehydration, infiltration and embedding were followed. Serial longitudinal and transverse sections were cut at a thickness of 2–5 μm and stained in Delafield's heamatoxylin. Voucher specimen number TP 2122 has been deposited in the Herbarium of Sri Krishnadevaraya University and Madras Herbarium, Coimbatore.

Observations Microsporangium, microsporogenesis and male gametophyte. Anther is tetrasporangiate. The youngest stage which we got showed a single row of pollen mother cells in longitudinal section (Fig. 1A). In transverse section, there is only one pollen mother cell. Hence each sporangium contained four pollen mother cells, while the anther contained sixteen pollen mother cells. Anther wall at this stage consists of epidermis, hypodermal layer, middle layer and tapetum (Fig. 1A). The epidermis persists in the mature anther as stretched cells. The endothecial cells develop fibrous thickenings and form fibrous endothecium (Fig. 1C). Anther tapetum is of the periplasmodial type (Fig. 1B). When one-nucleate pollen grains begin to develop exine, the walls of the tapetal cells break down and the cytoplasm flows in to the anther locule forming periplasmodial tapetum. The periplasmodium is consumed by the developing pollen

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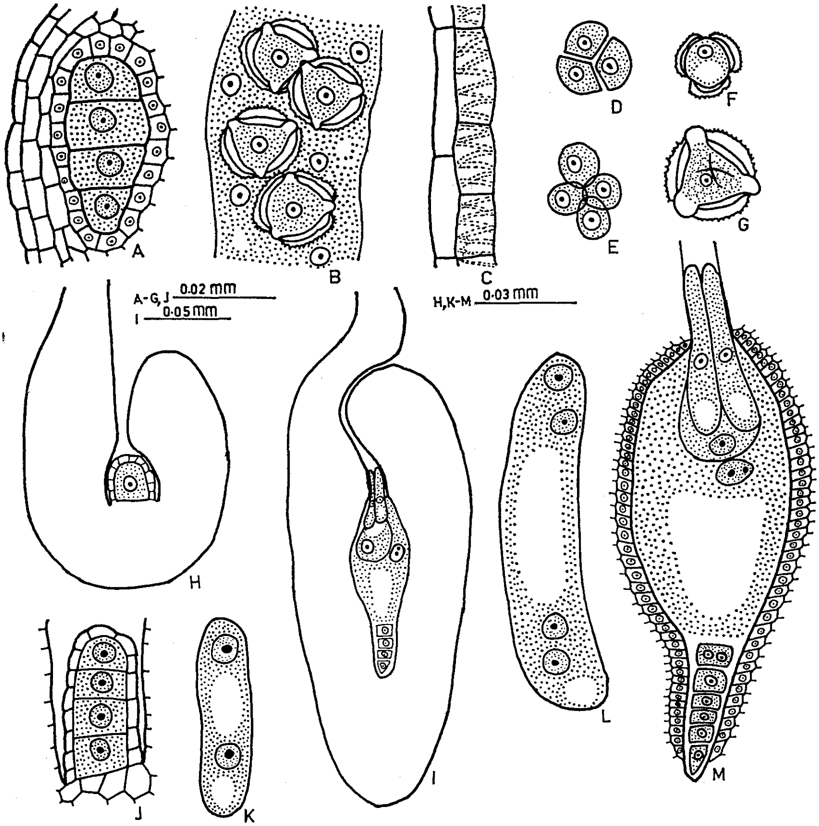

Fig. 1. *Gnaphalium polycaulon.* A. Longitudinal section of anther lobe showing pollen mother cells and wall layers. B. Longitudinal section of anther lobe showing 1-celled pollen grains and periplasmodial tapetum. C. Fibrous endothecium and epidermis. D, E. Tetrahedral and iso-bilateral pollen tetrads respectively. F. One-celled pollen grain. G. 3-celled pollen grain. H, I. Ovule at megaspore mother cell stage and organised embryo sac stage respectively. J. Megaspore tetrad. K. Two-nucleate embryo sac. L. Four-nucleate embryo sac. M. Organised embryo sac.

grains and no trace of it is left at maturity. Pollen mother cells undergo meiotic divisions resulting in tetrahedral and iso-bilateral pollen tetrads (Fig. 1D, E), the former being more prevalent. Microspores, soon after their release from the tetrads, enlarge and develop a thick exine (Fig. 1F, G). Pollen grains at the shedding are 3-celled with 3 germ pores (Fig. 1G).

Ovary and Ovule. The ovary as in other members of Compositae is bicarpellary, syncarpous and unilocular with a basal ovule. The ovule is anatropous, unitegmic and tenuinucellate (Fig. 1H, I). The tip of the integument is bulged and consequently forms the micropyle (Fig. 1I). The cells of inner epidermis of the integument at the time of megaspore tetrad formation enlarge radially, acquire dense cytoplasm and function as integumentary tapetum. It remains uniseriate with uninucleate cells throughout its further growth till it is com-

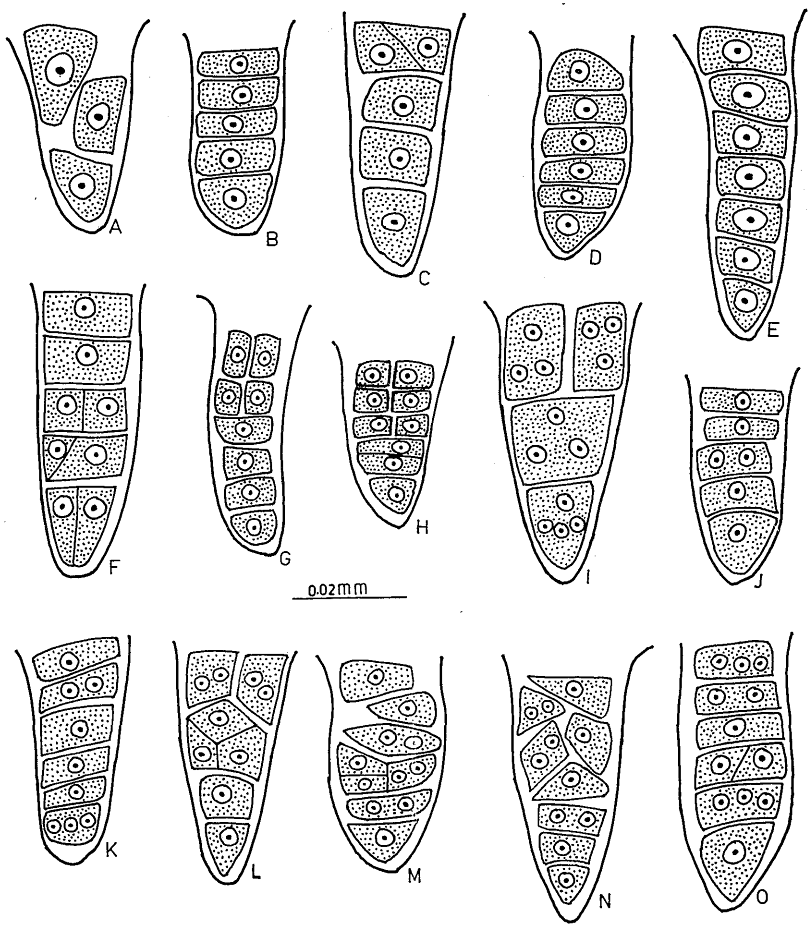

Fig. 2. *Gnaphalium polycaulon.* A–O. Antipodal cells.

pletely absorbed.

Megasporogenesis and femalé gametophyte. The single hypodermal archesporial cell directly functions as the megaspore mother cell. Meiosis I and II lead to the establishment of a linear tetrad of megaspores (Fig. 1J). The chalazal megaspore is functional while the micropylar three megaspores degenerate. The functional megaspore undergoes three nuclear divisions resulting in an 8-nucleate embryo sac of the *Polygonum* type. (Fig. 1K–M). The polar nuclei fuse near the egg apparatus forming secondary nucleus. The synergids elongate and

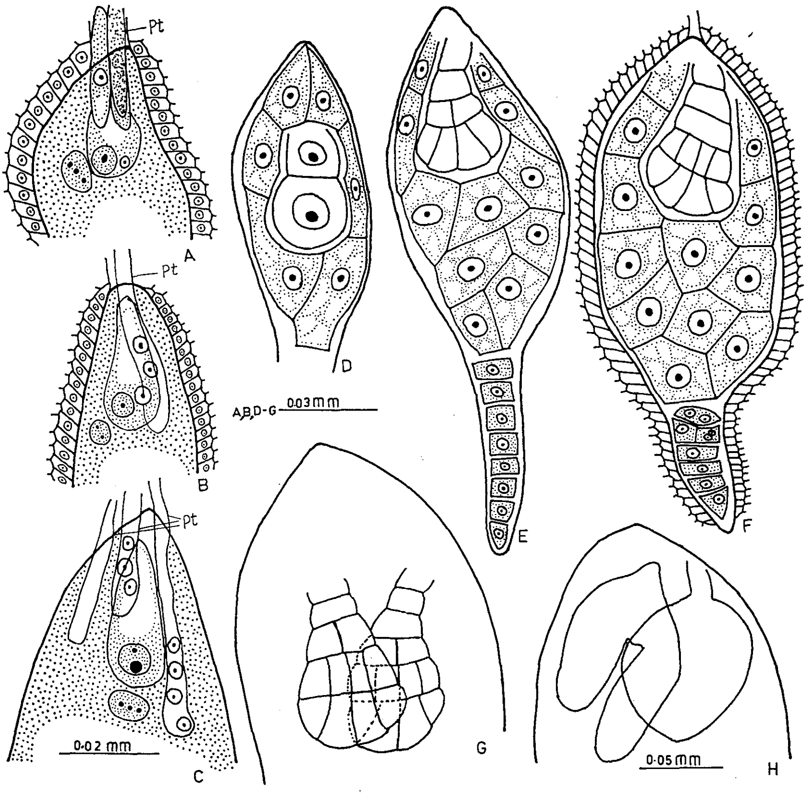

Fig. 3. *Gnaphalium polycaulon*. A. Micropylar part of the embryo sac showing fertilization. B. Micropylar part of the embryo sac showing pollen tube with three sperms. C. Microphylar part of the embryo sac showing zygote, triple fusion nucleus and three pollen tubes. Note three and four sperms in the pollen tubes. D–F. Stages in the development of the endosperm. G, H. Micropylar part of the embryo sac showing two embryos. pt, pollen tube.

protrude in to the micropyle forming synergid haustoria (Fig. 1M). Antipodal cells in the beginning are three in number and they are arranged nearly in a linear fashion (Fig. 2D). But during later stages of development the number of cells increase up to 9 (Fig. 2B-O). These antipodals are arranged linearly (Fig. 1B, D, E, J, K). But some of them are arranged in two rows or diagonally. In some instances the number of nuclei in the antipodal cells may increase (Fig. 2I-O) and as many as four nuclei have also been observed (Fig. 2I). Antipodal

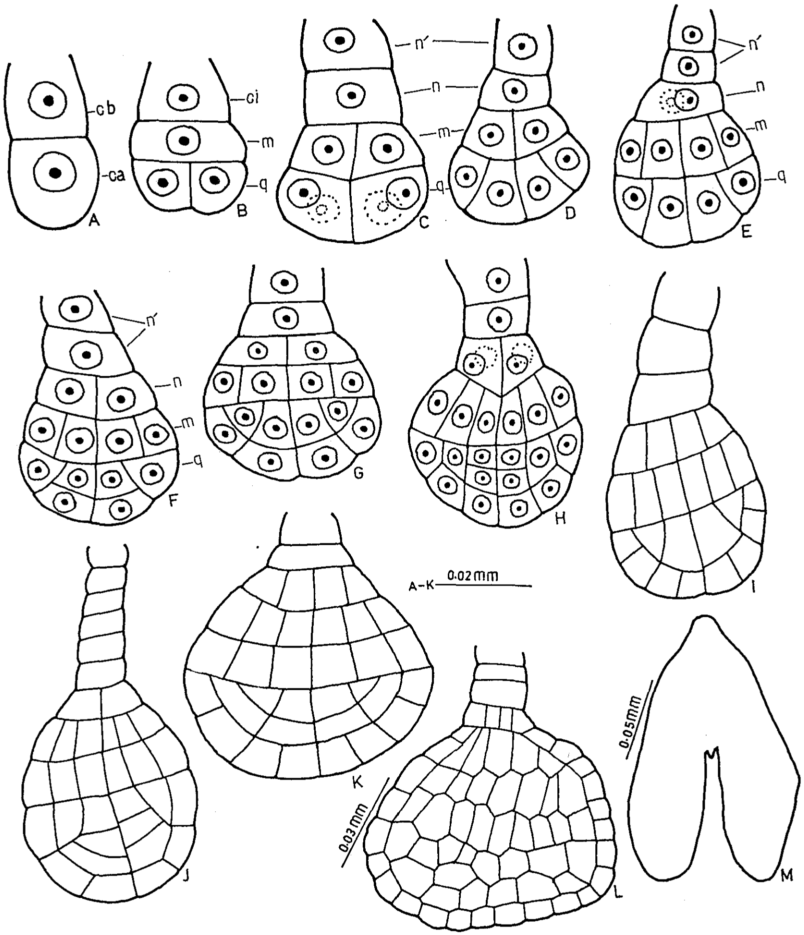

Fig. 4. *Gnaphalium polycaulon*. A-M. Stages in the development of embryo.

cells are persistent up to the globular stage of the embryo (Fig. 3F).

Fertilization, endosperm and embryo. Entry of the pollen tube is porogamous (Fig. 3A). Usually only one pollen tube enters the embryo sac. In Fig. 3C, where syngamy and triple fusion has already been completed, there are three pollen tubes, of which one has already discharged its contents, while the second and third pollen tubes possess 3 and 4 sperms respectively. The entry of additional pollen tube naturally results in the release of supernumerary male gametes inside the embryo sac. Rarely one and the same pollen tube may also carry more than two sperms (Fig. 3B, C). Thus two types of polyspermy has been recorded in *Gnaphalium polycaulon*.

Endosperm development is of the cellular type. First division of primary endosperm nucleus is followed by transverse wall and two cells are formed. Further divisions in these two cells take place in all directions and a massive tissue is formed filling the embryo sac with cellular tissue (Fig. 3D–F).

The first division of the zygote, which is transverse, results in a basal cell *cb* and a terminal cell *ca* (Fig. 4A). The former divides transversely engendering two superposed cells *m* and *ci*, while the latter (*ca*) divides vertically forming two juxtaposed cells (Fig. 4B). Thus a T-shaped 4-celled proembryo results at the end of second cell generation (Fig. 4B). Each of the terminal cells divide by vertical wall at right angles to the first wall (Fig. 4C) giving rise to the quadrant stage. At this stage the cell *m* divides vertically forming two juxtaposed cells while the cell *ci* undergoes transverse division resulting in *n* and *n′*.

The destination of the individual tiers and the development of embryo (Fig. 4A–M) is represented schematically in the following manner.

1. First cell generation.
   Proembryo consists of two cells disposed in two tiers:
   ca = pco + pvt
   cb = phy + icc + iec + co + s

2. Second cell generation.
   Proembryo consists of four cells disposed in three tiers:
   q = pco + pvt
   m = phy + icc
   ci = iec + co + s

3. Third cell generation.
   Proembryo consists of eight cells disposed in four tiers:

4. Fourth cell generation.
   Proembryo consists of sixteen cells disposed in five tiers:

q=pco+pvt	q=pco+pvt
m=phy+icc	m=phy+icc
n=iec+co	n=iec+co
n′=s	o=(1/2)s
	p=(1/2)s

Thus the embryo development follows Grand period I, megarchetype II, series A, sub series A_2 in the first group according to Souèges system. According to Johansen system (1950) it follows *Senecio* variation of Asterad type.

Polyembryony. Occasionally two embryos have been met with in the micropylar region of the embryo sac (Fig. 3G, H). Since more than one pollen tube have entered the embryo sac and also more than one sperm recorded from one pollen tube, the additional sperms might have fertilized with one of the synergids. This type of polyembryony according to Maheshwari (1950) is classified as true polyembryony.

Discussion In *Gnaphalium polycaulon* the synergids project beyond the embryo sac in to the micropyle and function as synergid haustoria. In the family Compositae synergid haustoria have earlier been recorded in *Ursinea anthemoides, U. chrysanthemoides, U. cakelifolia* (Dahlgren 1924, Schurhoff 1926, Vidayathi 1973, Ahlstrand 1978), *Cotula australis* (Davis 1961b), *Elephantus scaber* (Pullaiah 1979) and *Platycarpha carlinoides* (Ahlstrand 1979).

In the present study increase in the number of antipodal cells and the number of nuclei has been observed in *Gnaphalium polycaulon*. Multiple antipodals in the tribe Inuleae is not uncommon having been recorded in *Blumea laciniata* (Banerji 1942), *Podolepis jaceioides* and other species of *Podolepis* (Davis 1961a) and *Blumea membranacea* (Pullaiah 1979b).

Physical barriers usually prevent the entry of more than one pollen tube into the embryo sac. But in *Gnaphalium polycaulon* more than one pollen tube entering the embryo sac has been observed. Such a condition has earlier been recorded in *Eclipta prostrata* (Deshpande 1961) and *Caesulia axillaris* (Deshpande 1962). Some times same pollen tube may discharge more than one sperm resulting in polyspermy. Similar condition in the family Compositae has been recorded in *Crepis capillaris* (Gerassimova 1933), *Parthenium argentatum, P. incanum* (Dianova et al. 1935), *Taraxacum kok-saghyz* (Warmke 1943), *Melampodium divaricatum, Carthamus tinctorius* (Maheswari Devi &

Pullaiah 1976a, b) and *Crepis tectorum* (Gerassimova-Navashina 1952).

Additional sperms that entered the embryo sac may fertilize with the synergid resulting in polyembryony. Synergid embryos have earlier been recorded in *Crepis capillaris* (Gerassimova 1933), *Carthamus tinctorius* (Maheswari Devi & Pullaiah 1976) and *Eclipta prostrata* (Deshpande 1961).

One of us (V.R.) is thankful to the U.G.C. for the award of fellowship.

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Gnaphalium polycaulon の花粉・胚嚢・胚形成について報告する。花粉のタペータムのできかたは periplasmoidal type である。胚嚢形成は *Polygonum* type である。胚嚢の2個の助細胞は珠孔の中へ伸長して，吸収器の役割をもつ。3個の反足細胞は分裂して数を増し，9細胞にまでなる。この一部の細胞はしばしば多核となる。しばしば2個以上の精細胞が胚嚢に入ることや，多胚の現像が認められる。胚乳形成は cellular type である。胚形成は Johansen の分類の Asterad type の *Senecio* variation に一致する。

□IRVINE, D.E.G. & D.M. JOHN (ed.): **Systematics of the green algae: The systematics association special volume No. 27.** 449 pp. 1984. Academic Press, London. ¥25,330. 緑藻類の系統と分類に関する講演集である。クロロフィル a と b をもち，光合成によりでんぷんをつくる植物から，コケ類と維管束植物を除くすべてを含む緑藻類が，異質な系統群からなるらしいことは既に幾つかの観点から指摘されていたところであるが，最近の微細構造の研究はこの点をさらに浮彫りにしつつある。本書は20の講演をそれぞれ章として収録し，微細構造に基づく緑藻の系統と分類，緑藻の高次分類群の分類，緑藻の属の分類と細胞構造，緑藻の化学分類，緑藻の分類系の成立一過去と未来一の5部にまとめている。第1部は本書のハイライトともいうべきもので，微細構造上の形質，特に核分裂と細胞分裂の様式，鞭毛装置構造などに基づいた緑藻類の系統解析の試みと新分類系の提唱などを収録する。ここで K.R. Mattox & K.D. Stewart は従来の緑藻類（車軸藻類を含む）に下記の5綱を配している。I. ミクロモナス綱 Micromonadophyceae（緑色植物の祖先型を含むと考えられる群），II. 車軸藻綱 Charophyceae（陸上緑色植物系列緑藻），III. アオサ綱 Ulyophyceae（海の緑藻が主体），IV. プレウラストルム綱 Pleurastrophyceae（プラシノ藻の *Tetraselmis* などを含む），V. 緑藻綱 Chlorophyceae（狭義の緑藻類で，淡水の緑藻が主体）。近年，知見の急速な蓄積に伴い，“緑藻類の新しい分類”の声を聞くことが多くなったが，この方面の研究成果の現状を知りたい人にとって格好な書物である。 （千原光雄）